# DNA Barcoding Analysis of Marine Caridean Shrimps from Alaska

**David Drumm, Mike Canino, Troy Buckley, and Melanie Paquin**

Resource Assessment and Conservation Engineering Division, Alaska Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 7600 Sand Point Way NE, Seattle, Washington 98115, U.S.A.

## Introduction.

DNA barcoding is the application of short sequences of DNA to species identification and has become a powerful discipline since its inception (Hebert *et al.* 2003a,b), aiming to assess and document biodiversity at a quicker pace than traditional methods. One of the main advantages of this method is its ability to flag species that are potentially new to science (cryptic species). It also has advantages over morphological approaches when analyzing stomach contents and identifying larval forms and damaged specimens. Accurate species identification is critical for understanding their distribution and abundance and to inform ecosystem-based management. It has been effective in a variety of metazoan groups, including marine caridean shrimps (Costa *et al.* 2007; Radulovici *et al.* 2009; da Silva *et al.* 2011).

*Crangon* is a commercially and ecologically important genus of caridean shrimps, but its taxonomy is complicated by a lack of diagnostic morphological characteristics (Holthuis 1980; Luttikhuizen *et al.* 2008; Campos *et al.* 2012). This is especially true for *C. crangon, C. septemspinosa,* and *C. alaskensis*. *Crangon crangon* is found only in the Northeast Atlantic and *C. septemspinosa* is found in the Northwest Atlantic (type locality) and apparently the North Pacific, although some researchers do not recognize it occurring in the North Pacific (e.g. Squires 1990). *Crangon alaskensis* occurs in the Northeast Pacific and is distinguished from *C. septemspinosa* by possessing a very faint carina on the fifth abdominal segment. There is confusion whether these three species and *Crangon affinis* in the Northwest Pacific are actually the same species (Luttikhuizen *et al.* 2008). The genus reaches its greatest diversity in the Northeast Pacific, which is hypothesized to be its center of origin (Luttikhuizen *et al.* 2008). It has been demonstrated that it plays an important role in the ecosystem as prey and/or predator on juveniles of commercially important flatfishes (Campos *et al.* 2012). In an effort to document the diversity of Alaska's marine invertebrate fauna (Drumm *et al.,* in review), it became clear that the geographic ranges of many shrimp species are largely undocumented.

We tested the utility of barcoding approaches to identify candidate species of the marine shrimp genera *Crangon* and *Neocrangon* from Alaska and compared the newly generated sequences with sequences in GenBank and BOLD among congeners and conspecifics. Major goals were to analyze sequence divergence among conspecifics in different geographic regions to test for possible cryptic species and to help resolve taxonomic issues in problematic species.

## Materials and Methods.

### *Samples*

Shrimps were collected in the Chukchi Sea, Aleutian Islands, and Gulf of Alaska between 2005 and 2012 with most of the samples being collected in 2012 (Fig. 1). Samples were photographed and stored in 100% ethanol and morphological identifications were made following the keys in Butler (1980) and Vassilenko and Petryashov (2009).

### *DNA Extraction, PCR Amplification, and Sequencing*

Total genomic DNA was extracted from a small piece of abdominal muscle tissue using DNeasy Tissue Kits (Qiagen). A partial region of the COI gene (~ 700 bp) was amplified using three primer pairs depending on the taxa. Polymerase chain reaction (PCR) amplifications were carried out in 12.5 µL reactions and PCR products were sent to the DNA Sequencing Center at the University of Washington for purification and sequencing.

### *Data Analysis*

Sequence contigs from forward and reverse strands were assembled using Sequencher™ v. 5.1. Sequences were then aligned using ClustalW as implemented in BioEdit 7.0.5.3. Sequence divergences were calculated using the Kimura 2-parameter (K2P) distance method, and a neighbor-joining (NJ) tree was constructed using the bootstrap (BS) procedure with 10000 replications as implemented in MEGA 5.05. A haplotype accumulation curve with 95% confidence intervals was assembled using the program R-package SPIDER v1.1 to assess the amount of variability in our samples.

**Table 1.** Intra- (numbers in italic) and interspecific Kimura 2-parameter (K2P) genetic divergences for *Crangon* and *Neocrangon* species. The range of divergences between *C. septemspinosa* in the NW Atlantic and specimens identified as *C. septemspinosa* in the Chukchi Sea is highlighted in yellow: Chukchi Sea (CS), California (CA).

| | *C. septemspinosa* | *C. alaskensis* | *C.* sp (CS) | *C. angustimana* | *C.* sp (CA) | *C. crangon* | *C. dalli* | *N. communis* | *N. abyssorum* |
|---|---|---|---|---|---|---|---|---|---|
| *C. septemspinosa* | *0.0 – 0.9%* | | | | | | | | |
| *C. alaskensis* | 7.6 – 8.8% | *0.0 – 0.3%* | | | | | | | |
| *C.* sp. (CS) | 8.3 – 10.0% | 6.0 – 7.1% | *0.0 – 0.5%* | | | | | | |
| *C. angustimana* | 8.0 – 9.1% | 6.8 – 6.9% | 6.7 – 7.1% | *0.0%* | | | | | |
| *C.* sp. (CA) | 11.3 – 12.5% | 7.9 – 8.1% | 8.7 – 9.1% | 9.4% | – | | | | |
| *C. crangon* | 8.1 – 9.5% | 6.4 – 6.8% | 5.8 – 6.6% | 5.6 – 5.9% | 7.6 – 8.0% | *0.0 – 0.5%* | | | |
| *C. dalli* | 14.2 – 15.6% | 13.2 – 14.0% | 12.9 – 13.6% | 13.4 – 13.6% | 14.4% | 11.9 – 12.7% | *0.0%* | | |
| *N. communis* | 19.2 – 23.6% | 17.3 – 19.8% | 15.8 – 19.2% | 17.7 – 20.1% | 20.2 – 22.0% | 18.2 – 20.6% | 18.3 – 18.9% | *0.3 – 6.9%* | |
| *N. abyssorum* | 23.4 – 24.6% | 23.9 – 24.6% | 22.7 – 24.3% | 21.6 – 22.0% | 23.9% | 19.7 – 21.0% | 22.5 – 22.6% | 17.9 – 20.2% | *0.3%* |

## Results and Discussion.

The mean intraspecific divergences were less than 1% except for *Neocrangon communis*, which was 4%. This was due to the Chukchi Sea specimen differing by as much as 6.9% from the British Columbia specimens (Table 1). A barcode gap was still evident, as it diverged from the only congeneric species analyzed by over 15% (Table 1). Whether this gap holds up will have to await until additional congenerics are analyzed. *Neocrangon* forms a monophyletic group in the NJ tree (BS = 92%) (Fig. 2). *Crangon dalli* had the highest interspecific divergences (all over 10%) and did not show any intraspecific variation (Table 1). The lowest interspecific divergence was seen between *C. crangon* and *C. angustimana* (mean 5.7%). Specimens identified as *C. septemspinosa* from the Chukchi Sea diverged from topotypic specimens in the Northwest Atlantic by 9.1% [represented as *Crangon* sp. (CS) in Table 1 and Fig. 2 and highlighted in yellow] and likely represent a new species.

Based on our results, it appears that *Crangon septemspinosa* may not occur in Alaskan waters. A specimen identified as *C. septemspinosa* by one of us (Drumm) from the Gulf of Alaska grouped with two specimens of *Crangon alaskensis* from British Columbia (highlighted in blue in Fig. 2) and was likely misidentified. *Crangon septemspinosa* is very similar to *C. alaskensis* but lacks a keel on the fifth abdominal segment, which when present can be very faint and hard to see. Another COI sequence (GenBank Accession #AF125416.1) that came from a specimen identified as *C. septemspinosa* grouped with *C. crangon* on the NJ tree [shown as *Crangon* sp. (CA)], but without bootstrap support. This specimen was purchased at a marine supply company in California, but it is unknown where the specimen actually came from (Shank *et al.* 1999).

It must be pointed out that we confirmed a misidentification in the Barcode of Life Database (BOLD). Two specimens in BOLD identified as *Crangon alaskensis* are actually *Crangon dalli.* Misidentifications are a major problem when using reference libraries in online databases and a serious limitation to the utility of DNA barcoding. The haplotype accumulation curve revealed that the genetic diversity has not been fully sampled, as indicated by the steep slope and lack of an asymptote (Fig. 3).

In conclusion, DNA barcoding proved to be a useful tool for the identification of marine caridean shrimps of Alaska. It also led to the discovery of a possible cryptic species in the Chukchi Sea, warranting further study to determine if there are any additional differences (i.e., morphological, ecological, etc.).

### Acknowledgments

**We would like to thank Greg Jensen (University of Washington) for his help with shrimp identifications and Jay Orr (NOAA-AFSC) for commenting on an early version of this poster. This project was funded by the North Pacific Research Board, grant number 1220.**

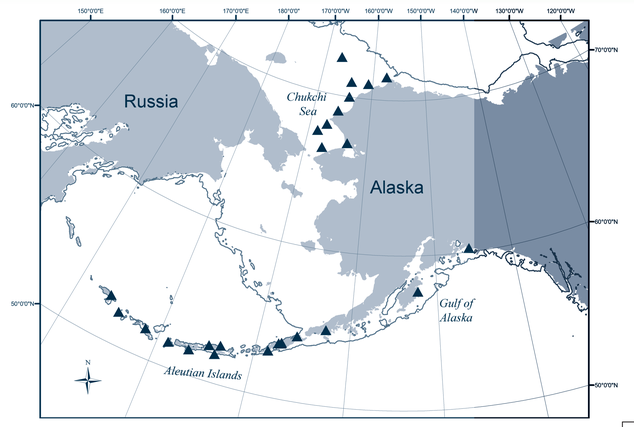


**Figure 1.** Map showing collecting localities (bold triangles) in the Chukchi Sea, Aleutian Islands, and Gulf of Alaska.

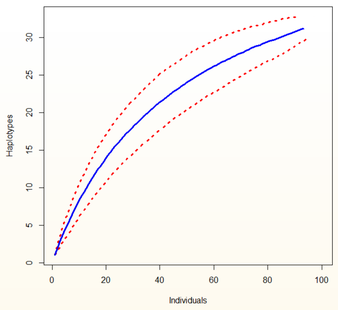


**Figure 3.** Haplotype accumulation curve. Mean (solid blue) and 95% confidence intervals (dotted red) are given.

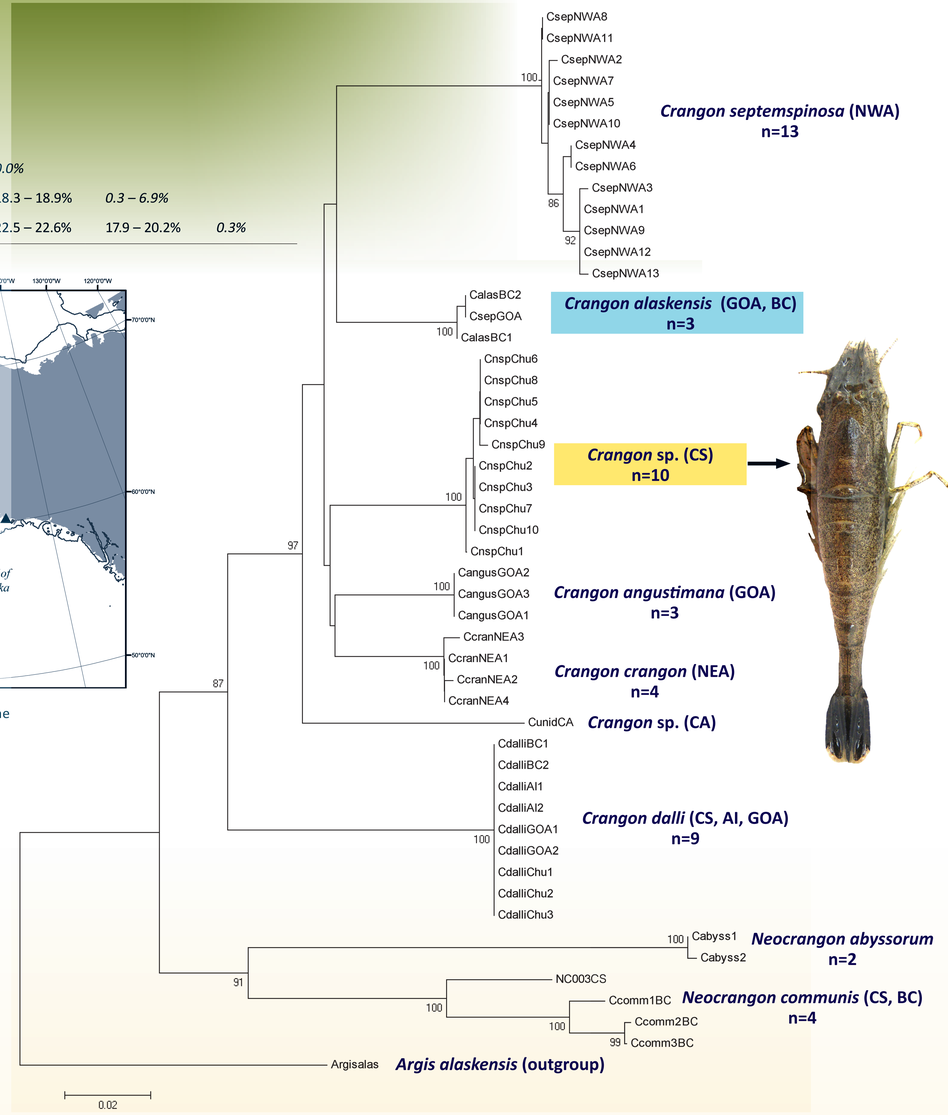


**Figure 2.** Neighbour-joining tree based on mitochondrial cytochrome *c* oxidase I gene sequences (K2P model). Bootstrap values above 85 based on 10000 replications are included. Specimens identified as *Crangon septemspinosa* in the Chukchi Sea are highlighted in yellow: Aleutian Islands (AI), British Columbia (BC), California (CA), Chukchi Sea (CS), Gulf of Alaska (GOA), Northeast Atlantic (NEA), Northwest Atlantic (NWA).

### References

Butler TH (1980) Shrimps of the Pacific coast of Canada. *Canadian Bulletin of Fisheries and Aquatic Sciences*, **202**, 1-280.

Campos J, Moreira C, Freitas F, van der Veer HW (2012) Short review of the eco-geography of *Crangon*. *Journal of Crustacean Biology,* **32**, 159-169.

Costa FO, deWaard JR, Boutillier J, Ratnasingham S, Dooh RT, Hajibabaei M, Hebert PDN (2007) Biological identifications through DNA barcodes: the case of the Crustacea. *Canadian Journal of Fisheries and Aquatic Sciences,* **64**, 272-295.

da Silva JM, Creer S, dos Santos A, Costa AC, Cunha MR, Costa FO, Carvalho GR (2011) Systematic and evolutionary insights derived from mtDNA barcode diversity in the Decapoda (Crustacea: Malacostraca). *PLoS ONE,* **6**, 1-15.

Drumm, DT, Maslenikov, KP, VanSyoc, RJ, Orr, JW, Lauth, RR, Stevenson, DE, Pietsch, TW (in review) An annotated checklist of the marine macroinvertebrates of Alaska. *NOAA Professional Papers*, 942 pp.

Hebert PDN, Cywinska A, Ball SL, DeWaard JR (2003a) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London, B,* **270**, 313-321.

Hebert PDN, Ratnasingham S, deWaard JR (2003b) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London Series B,* **270**, S96-S99.

Holthuis LB (1980) FAO species catalogue. Vol. 1. Shrimps and prawns of the world. An annotated catalogue of species of interest to fisheries, Rome, Italy.

Luttikhuizen P, Campos J, Bleijswijk J, Peijnenburg KTCA, van der Veer HW (2008) Phylogeography of the common shrimp, *Crangon crangon* (L.) across its distribution range. *Molecular Phylogenetics and Evolution*, **46**, 1015-1030.

Radulovici AE, Sainte-Marie B, Dufresne F (2009) DNA barcoding of marine crustaceans from the Estuary and Gulf of St. Lawrence: a regional-scale approach. *Molecular Ecology Resources,* **9** (Suppl. 1), 181-187.

Shank TM, Black MB, Halanych KM, Lutz RA, Vrijenhoek RC (1999) Miocene radiation of deep-sea hydrothermal vent shrimp (Caridea: Bresiliidae): Evidence from mitochondrial cytochrome oxidase subunit I. *Molecular Phylogenetics and Evolution,* **13**, 244-254.

Squires HJ (1990) Decapod Crustacea of the Atlantic coast of Canada. *Canadian Journal of Fisheries and Aquatic Sciences,* **221**, 1-532.

Vassilenko SV, Petryashov VV (eds.) (2009) Illustrated Keys to Free-Living Invertebrates of Eurasian Arctic Seas and Adjacent Deep Waters, Vol. 1. Rotifera, Pycnogonida, Cirripedia, Leptostraca, Mysidacea, Hyperiidea, Caprellidea, Euphausiacea, Dendrobranchiata, Pleocyemata, Anomura, and Brachyura. Alaska Sea Grant, University of Alaska Fairbanks, 1-186.



NOAA FISHERIES